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*The sterilizing efficiency of the
Alfa-Laval Vacu-Therm Instant
Sterilizer*

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The sterilizing efficiency of the Alfa-Laval Vacu- Therm Instant Sterilizer

(Steriliseringseffekten hos Alfa-Laval Vacu-Therm
Instant Sterilizer)

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Introduction

The Alfa-Laval Vacu-Therm Instant Sterilizer (VTIS) is a milk sterilizing plant based on a continuously operating ultra-high temperature procedure. After forewarming in a plate heat-exchanger the milk is heated to 130–140° C by injection of steam. The milk is then cooled rapidly by partial evaporation in vacuum. The holding time at the high temperature is three to four seconds. The evaporation causing the fast cooling is so regulated as to restore exactly the original dry matter content of the milk. More detailed information on the plant and its performance has been published by ARPH and HALLSTRÖM (1). Their bacteriological results indicate that the bactericidal efficiency of the procedure is sufficient for its purpose. It is the purpose of this paper to present some further information on this aspect of the VTIS plant.

The study forms part of a larger scheme of investigations into various problems of milk sterilization and aseptic packaging of sterile milk. These investigations were planned and performed by the Dairy Department of the Alnarp Institute in co-operation with the State Dairy Research Station, Alnarp, and the Alfa-Laval and Tetra Pak Companies. Other results of these investigations have previously been published (1, 20, 22).

The bacteriological investigation presented here was made at the State Dairy Research Station. The VTIS plant was operated by Messrs Holm and Stenqvist of the Alfa-Laval Co.

Previously other UHT sterilizers have been investigated from this point of view and the practical and theoretical problems involved have been discussed. These problems concern the difficulty to detect and enumerate the few bacteria possibly present in the sterilized milk under normal conditions, to avoid contamination during sampling, to express and evaluate the results obtained, and to select appropriate test organisms.

GALESLOOT (8, 9) used milk artificially infected with a large number of bacterial spores. He thus avoided the difficulties connected with the determination of very low spore counts. He mentions no special difficulties as regards the aseptic sampling. For the determination of the spore count he used a dilution technique.

WILLIAMS *et al.* (23) made experiments with a suspension of spores in water. For the determination of the number of surviving spores a membrane filter method was used. When working with milk these same authors (FRANKLIN *et al.*, 5, 7) used the technique proposed by GALESLOOT (8).

HOSTETTLER (12, 13) gives no information as to the testing technique used in his investigation on the uperization but the same UHT plant was later investigated by WÄLZHOLOZ *et al.* (24). These authors used a direct plate count method for the detection of surviving organisms in 1 ml samples.

For the evaluation of the bacteriological results, most investigators use the assumption of the logarithmic thermal destruction of spores. BURTON (2, 3) relates the thermal death time and temperature characteristics of bacterial spores to the integrated heat treatment in the heating and holding sections. His general procedure for the calculation of the sporicidal efficiency was used successfully for predicting the efficiency of the APVHX sterilizer (7). Thereby the bacteriological investigations of FRANKLIN *et al.* (5, 6) were used. This method would seem convenient for guidance in the manufacturing designing of new sterilizers (and pasteurizers as well). The testing of a particular plant, however, would still seem likely to demand also practical microbiological experiments. The procedure described by GALESLOOT (8) and in principle also employed by FRANKLIN *et al.* (5, 7) seems convenient for this purpose. The sterilizing efficiency was defined by GALESLOOT (8) as follows:

$$\text{sterilizing efficiency} = \log \frac{\text{initial spore count}}{\text{final spore count}}$$

As regards the choice of test organisms to be used in the determination of the sterilizing efficiency, the various investigations differ but little. In most cases *Bac. subtilis* and *Bac. stearothermophilus* have been used. *Bac. subtilis* is a normal milk inhabitant, frequently found in raw milk supplies and also as a spoilage organism in processed milk and sterile milk (RIDGWAY, 19, GRINSTED and CLEGG, 10, FRANKLIN *et al.*, 4). *Bac. stearothermophilus* was isolated from milk by SMITH *et al.* (21). Its main merit in connection with the testing of milk sterilizers, however, seems to be the extremely high heat resistance of its spores. From the data presented by MURRELL (18) the following may be cited here.

Species	Time in minutes for 99 % killing at 121° C
<i>Bac. cereus</i>	0.013
<i>Bac. megatherium</i>	0.08
<i>Bac. subtilis</i>	0.16
<i>Bac. spec.</i> (strain 320)	6
<i>Bac. stearothermophilus</i>	28

On the basis of these considerations the procedure outlined by GALESLOOT (8) was adopted for the present investigation. Raw whole milk was inoculated with suspensions of spores of *Bac. subtilis* and *Bac. stearothermophilus*. The milk was run through the plant at various sterilizing temperatures, the highest being used first. Samples of the raw inoculated milk and of the milk after heat treatment were taken and the number of spores present was determined. The following details were observed.

Experimental methods

Test organisms

The test organisms were *Bac. subtilis*, NCDO 736 (ATCC 9524) and *Bac. stearothermophilus*, NCDO 733 (1518; ATCC 7953).

Bac. subtilis was grown for 24 hours at 30°C in the medium used by HALVORSON (11) in his work on rapid and simultaneous spore formation. The culture was then warmed to 76°C for ten minutes and cooled again. Fresh broth was inoculated from the heated culture, a 10 % inoculum being used. After incubation for four hours a new transfer to fresh broth was made. This last culture was incubated for about three hours and was then used for sawing plates of a solid medium (the same broth with 1.5 % agar). The plates were incubated for two days, the cells and spores were washed off the agar surface with 0.9 % saline filtered through cotton, centrifuged and washed three times with water and finally suspended in a buffer solution according to MORRISON and RETTGER (17). The suspension was kept in a refrigerator until used.

The heat resistance of spores harvested on different occasions was compared. The suspensions diluted with buffer at pH 6.9 were heated to 85°C. Samples were taken at certain intervals for determination of the number of surviving bacteria. The results summarized in Table 1 indicate fairly great variations in heat resistance among different harvests.

Table 1. *The heat resistance of spores of Bac. subtilis 736, harvested on various occasions. The heat resistance is expressed as the number of surviving spores in per cent of the initial number.*

Heating time hours	1	2	3	4
1	28.0	73.6	18.5	29.3
2	9.6	20.8	4.0	0.48
3	0.18	8.5	0.8	0.012
4	0.15	2.6	0.16	0.017
5	0.08	0.6	0.04	0.017

For a similar comparison spore suspensions were also prepared by the same technique using MSA agar plates according to GRINSTED and CLEGG (10) and gypsum plates as proposed for this purpose by HOSTETTLER (13). Table 2 shows that the HALVORSON medium gave spores slightly more heat resistant than the other two media.

Table 2. The heat resistance of spores of *Bac. subtilis* 736, grown on different media. The heat resistance is expressed as the number of surviving spores in per cent of the initial number.

Heating time hours	MSA	HALVORSON'S medium + agar	Gypsum plate	HALVORSON'S medium + agar
1	20.0	28.0	64.3	73.6
2	2.1	9.6	26.5	20.8
3	0.14	0.18	6.4	8.5
4	0.02	0.15	0.95	2.6
5	0.006	0.08	0.07	0.6

Bac. stearothermophilus was grown at 50°C, which was the highest temperature compatible with a reasonably good spore formation. The HALVORSON medium was used. A first culture was grown for 24 hours and was then heated to 76°C for ten minutes. Fresh broth was then inoculated from the heated culture, the inoculum being the 10 %. After incubation for 24 hours this culture was used for inoculation of the final broth in which the organism was kept for seven days before the cells and spores were harvested by centrifugation. The spores were washed and transferred to buffer as above. The suspension was kept in a refrigerator until used (for the experiment).

The heat resistance of the spores was not found to be substantially increased by culture on gypsum plates as claimed by HOSTETTLER (13) (Table 3). As this method moreover failed to give sufficiently large yields, the method was not used.

Table 3. The heat resistance of spores of *Bac. stearothermophilus* 733, grown on gypsum plates and HALVORSON'S medium. The heat resistance is expressed as the number of surviving spores in per cent of the initial number.

Heating time hours	Gypsum plate	HALVORSON'S medium
2	42.9	10.0
3	3.4	9.0
4	0.43	6.2
5	0.14	5.8

Milk sterilization and sampling

The spore suspension was mixed into raw milk (fat content 3 %) in a storage tank connected with the VTIS plant. The raw inoculated milk was sampled from this tank.

Before starting the milk sterilization the VTIS plant was sterilized according to the regular procedure for this plant. The sampling devices described below had previously been connected to the sampling valves of the milk outlet tube. The sterile plant was started with recirculating water at over 140° C. The water was followed by the milk and the sterilizing temperature was gradually lowered to the upper one of the temperatures to be tested. When a steady state had been reached samples were taken. The sterilizing temperature was then lowered to the next level, samples were taken and the procedure was repeated according to plan.

The temperatures were 140°, 135°, 130°, 125° and 120° C. The holding time at these temperatures was three to four seconds in all cases.

For the sampling of the sterilized milk four valves had been inserted into the milk pipe after the cooler. Each of these valves was connected with a set of flasks with the aid of a series of rubber tubings, T-tubes and clamps. The arrangement was such as to allow the taking of four samples of 100 ml, and three samples of 1000 ml from each valve. Before connecting the rubber tubes to the sampling valve the whole assembly was sterilized in an autoclave.

Bacteriological analysis

The initial spore count in the inoculated raw milk was determined after heating the sample to 76° C for ten minutes. Suitable dilutions were prepared using sterile milk as a dilution fluid.

The samples of sterilized milk in the size of 100 ml and 1000 ml were used directly. From one of the flasks smaller samples were taken in the laboratory to make samples of 10 ml, 1 ml and the diluted samples.

All samples and dilutions were stored for seven days at 30° C in the case of *Bac. subtilis*, and at 50° C in the case of *Bac. stearothermophilus*.

The samples were then investigated as follows. All samples that showed spoilage were controlled in microscope for the presence of rods and spores. The remaining samples were investigated microscopically and by plating on the MSA medium. If both tests were negative, the sample was considered sterile.

The most probable number of surviving organisms was calculated with the aid of McCrady's (16) tables.

Results and discussion

The results of the experiments with whole milk and *Bac. subtilis* as a test organism are collected in Table 4.

Table 4. The sterilizing efficiency of the VTIS plant as found with *Bac. subtilis*.

Sterilizing temperature °C	Initial spore count per ml	Final spore count per ml	Logarithmic reduction
140	450,000	< 0.0004	> 9
135		< 0.0004	> 9
130		0.0007	8.8
125		0.45	6.0
135	4,000,000	< 0.0004	> 10
130		< 0.0004	> 10
125		0.04	8.0
120		4.5	6.0
135	75,000,000	< 0.0004	> 11
130		0.025	9.5
125		4.5	7.2
120		11.0	6.8

The three experiments gave fairly consistent results. As could be expected, the number of surviving organisms decreased with increasing sterilizing temperature and decreasing initial spore count.

The lowest number of surviving organisms that could be determined by the dilution technique used was 0.0004 spores per ml. If this figure is used, the logarithmic reduction is found to be more than nine in the temperature range of 135° to 140° C.

A sterilizing temperature of 130° C gave a logarithmic reduction of about nine, whereas 125 and 120° C were somewhat less efficient.

The results of the corresponding experiments with *Bac. stearothermophilus* are reported in Table 5.

The two runs with this test organism gave somewhat different results, again indicating the differences in heat resistance of the spores of different harvests.

In both cases, however, the logarithmic reduction was above seven for a sterilizing temperature of 135° C and higher. 120° and 125° C gave a fairly low reduction.

The experiments confirm the earlier findings that spores of *Bac. stearothermophilus* are more heat resistant than spores of *Bac. subtilis*.

It is of some interest to compare the results obtained for the Alfa-Laval VTIS plant with those reported for other UHT sterilizers.

Table 5. *The sterilizing efficiency of the VTIS plant as found with Bac. stearothermophilus.*

Sterilizing temperature °C	Initial spore count per ml	Final spore count per ml	Logarithmic reduction
140	10,000	< 0.0004	> 7.4
135		< 0.0004	> 7.4
130		0.45	4.3
125		1.4	3.8
135	250,000	< 0.0004	> 9
130		0.095	6.4
125		4	4.8
120		25	4.0

FRANKLIN *et al.* (5, 7) studied the sporicidal efficiency of the APVHX heat-exchanger. They found that a temperature of 135° C gave a logarithmic spore reduction of more than ten (calculated from their figures) in the case of *Bac. subtilis*. Using *Bac. stearothermophilus* they found it necessary to use 142° C to effect a reduction of seven.

GALESLOOT (8) who studied the sporicidal performance of the Stork tubular UHT sterilizer using *Bac. subtilis*, found a logarithmic reduction of more than six at a sterilizing temperature of 135° C.

HOSTETTLER (12, 13) states that 150° C gives a sufficient margin of safety for the "Uperisator". He used *Bac. stearothermophilus* as a test organism but gives no further particulars.

In conclusion it would seem as if the sporicidal efficiency of the various UHT sterilizers compared very well.

It now remains to discuss the sterilizing temperature to be used in practice in the case of the VTIS plant. With regard to the flavour of the milk and its physico-chemical properties the mildest possible heat treatment should be applied. On the other hand a sufficient margin of safety must be maintained with regard to sterility.

In the following discussion it is understood that the presterilization of the unit is efficient and that no re-infection of the sterilized milk occurs. Factors to be taken into account are then:

1. The "degree of sterility" considered as necessary (e.g. one non-sterile in 10,000, in 1,000 or in 100 units, bottles or packages);
2. The number of spores in the raw milk and their heat resistance; and
3. The possibility for any surviving spores to germinate and grow in the sterilized milk and spoil it.

The "degree of sterility" required depends upon the time necessary for a non-sterile unit to deteriorate beyond palatability and upon the rate of distribution and consumption. With a low rate of bacterial growth and an early consumption of all or most units of a batch, the demand on sterility may be low. If, on the other hand, the distribution and consumption of the milk takes a long time, any failures in the sterilization are likely to cause trouble and a high efficiency on the part of the sterilizer is imperative.

In this paper (as in GALESLOOT, 8, and SWARTLING and LINDGREN, 22) a "degree of sterility" corresponding to not more than one non-sterile unit out of 1,000 is assumed to meet the average demands. In some cases this figure will provide a considerable margin of safety, in other cases it may not prove sufficient. Anyhow, to meet this requirement a sterilizing efficiency corresponding to a logarithmic reduction of six to nine is necessary, the relevant figure being dependent upon the initial spore count and the volume of the packages. For one litre units the following relationship is valid.

Initial spore count per ml	Logarithmic reduction necessary
1	6
10	7
100	8
1,000	9

According to most workers in this field (for a summary review see JAYNE-WILLIAMS and FRANKLIN, 15) the spore content of bulk milk is higher in winter than in summer, and, furthermore, it varies with the sanitary conditions of milk production. Unfortunately, very little information is available concerning the order of magnitude of the spore count in milk in various countries. From the Netherlands, Great Britain and Australia figures have been reported for bulk milk ranging between 1-5 per 100 ml and 40-9,000 per 100 ml. Out of these spores only a small proportion belonged to heat resistant thermophilic spore formers. The majority of strains isolated belongs to *Bac. subtilis* or *Bac. licheniformis*.

The third factor to be considered concerns the conditions for the germination and growth in the sterilized milk of any possibly surviving spores. Little information is available on the UHT sterilized milk as a growth medium for spore formers. JAYNE-WILLIAMS (14) found some evidence of inhibition of heated spores in such milk. This aspect should be investigated further. In the present investigation, as in similar studies, it is not possible to distinguish between a truly sterile sample and a sample with a few surviving spores, which for one reason or another are not able to germinate and increase in number. This fact does not influence the conclusions drawn here.

From a practical point of view the temperature prevailing during the storage and distribution of the milk is of great importance. The influence of this factor can be fairly well predicted from the temperature characteristics of growth of the relevant bacteria. On the whole the higher temperature, the better growth; for mesophiles in the range 12° to 37° C and for thermophiles from 37° to 55° C. Under temperate climatic conditions only the mesophiles have to be considered.

Generalizing this information, one arrives at the conclusion that the VTIS should be run at a sterilizing temperature which gives a logarithmic reduction of seven to eight, or even slightly lower if smaller packages than one litre are being used.

From the experiments reported in this paper it is evident that in the VTIS plant sterilizing temperatures above and including 135° C result in a logarithmic reduction of the relevant order of magnitude. It should be borne in mind, however, that the experiments were performed at a spore density very much higher than that met with in practice. Consequently the adoption of the experimental findings for practical purposes involves an extension of the first order death curve to comparatively low initial spore counts, where this mathematical relationship may not longer be valid. Furthermore, the spores used in the experiments were prepared in the laboratory and their heat resistance may or may not equal that of natural spores.

It should further be observed that a higher temperature must be used when the initial spore count of the raw milk is higher than that assumed above and when conditions of storing, distribution and consumption of the milk are known to be severe.

Summary

The sterilizing efficiency of the Alfa-Laval Vacu-Therm Instant Sterilizer was studied. Raw whole milk was inoculated with suspensions of spores of *Bac. subtilis* and of *Bac. stearothermophilus*. The milk was run through the plant at differing sterilizing temperatures. Samples were taken of the raw inoculated milk and of the milk after heat treatment, and the number of spores present was estimated by means of the dilution technique. With *Bac. subtilis* spores the VTIS plant gave a logarithmic reduction of the order of nine or higher when the sterilizing temperature was kept at 130–140° C. With the more heat resistant *Bac. stearothermophilus* spores a logarithmic reduction of more than seven was effected at these temperatures. The practical significance of these findings are evaluated and it is concluded that for normal conditions a sterilizing temperature of 135° is appropriate with the VTIS plant. Under adverse conditions as regards the spore content of the raw milk, storing temperature and distribution, a higher temperature has to be used.

Sammanfattning

Steriliseringseffekten hos en Alfa-Laval Vacu-Therm Instant Sterilizer har undersökts. Rå helmjölk ympades med en sporsuspension av *Bac. subtilis* respektive *Bac. stearothermophilus*. Mjölken steriliserades vid olika temperaturer. Provtogs på den ympade råa mjölken och på den värmebehandlade mjölken. Sporantalet i de olika mjölkproven bestämdes med hjälp av en utspädningsteknik. Med *Bac. subtilis*-sporer erhöles i VTIS-anläggningen en logaritmisk reduktion av nio eller högre, när steriliseringstemperaturen var 130–140° C. Med den mera värmeresistenta *Bac. stearothermophilus* erhöles en logaritmisk reduktion av mer än sju vid dessa temperaturer. Den praktiska betydelsen av dessa resultat diskuteras, och slutsatsen blir, att för normala förhållanden en temperatur av 135° C är tillräcklig för en VTIS-anläggning. Under ogynnsamma förhållanden beträffande antalet sporer i den råa mjölken samt beträffande förvaringstemperatur och distribution måste högre temperatur användas.

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